A GELATIN-HARDENING BACTERIUM.

(Bacillus indurans, n.sp.*)*

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Bacteria which soften and liquefy gelatin are very numerous, but a microbe that hardens gelatin is, I think, a novelty. Such a micro-organism was isolated during the bacteriological examination of the tissues of *Schinus molle*, the specimen of which was exuding small quantities of a turquoise-coloured gum-resin. Two bacteria were isolated; one of them, from the bacterioscopic characters, appeared to be closely allied to, if not identical with, *Bact. acaciæ* var. *metarabinum*, and there its interest ends. The other produced a slime,* but not in sufficient quantity to warrant one in considering it to be of any importance on that account. When it was grown upon ordinary glucose-gelatin in stroke culture, the medium was slowly darkened in colour to a deep reddish-brown or mahogany colour. There were no signs of liquefaction, but on the contrary when the culture tube was put into a beaker of water and the water boiled, the gelatin maintained its sloped position, which prolonged boiling did not alter. The original medium would have liquefied soon after a temperature of 25° had been reached. There was thus something secreted by

* The slime was grown upon levulose-asparagin-tannin agar, and from it a gum was prepared in the manner that has been frequently described. The gum was coagulated by alcohol, basic and ammoniacal lead acetates, barium hydrate and copper sulphate followed by potassium hydrate. Tannin gave a slight opalescence. No reaction was obtained with copper sulphate, ferric chloride, neutral lead acetate, or ferric chloride followed by ammonia.

the bacterium that was capable of diffusing through gelatin and either directly or indirectly affecting it. The darkening of the medium was probably a phenomenon connected with the hardening.

Bearing in mind the action of tannin upon gelatin, I thought it possible that some of the constituents of the medium might have been altered to tannin and the darkening caused by traces of iron salts. This, however, did not prove to be the case, for the addition of ferric chloride to a tube of the molten medium before the hardening had proceeded far showed no reaction. Fluid cultures contained no formaldehyde, so that this substance is probably absent in the cultures on solid media.

Believing that the mahogany colour might be caused by an oxidase acting upon traces of tyrosin in the medium, I added tyrosin to nutrient gelatine and at the same time tried the effect of different sugars, etc., and noted the appearances at the end of 9 and 28 days. With saccharose, mannit, lactose and levulose the growths were good, the medium slowly liquefied, and only a trace of colour was produced. With tyrosin the nutrient gelatin showed a slight tinge of brown in the upper layers, but the colour disappeared. With galactose, dextrose, and dextrose-tyrosin, the growth was scanty and the medium became deep brown in colour, especially in the middle layers; there was a very slight liquefaction; a single drop of fluid rested at the lower end of the stroke on the 28th day. As no increase of colour resulted from the addition of tyrosin to dextrose, and as the colour that developed in the tube with tyrosin-nutrient gelatin was faint, it is probable that the production of colour is not due to the formation and oxidation of tyrosin by the bacterium. Oxidising enzymes were sought for by adding an alcoholic solution of gum guiacum to the glucose-gelatin before sterilisation. The medium, after infection, behaved as glucose-gelatin; the brown colour was obtained without a trace of blue, which is characteristic of the oxidising enzymes.

The partial liquefaction of the gelatin leads us to infer that there are two agents at work, one hardening, the other liquefying

the gelatin. In the presence of dextrose or galactose the latter is almost overcome by the former, but with other sugars the hardening and darkening substance is not produced. When the gelatin is hardened, the growth is always scanty, as if the active substance were an antiseptic like formaldehyde.

The hardening substance does not appear to be formed in fluid cultures, for when various cultures of the bacterium, such as Hansen's fluid, meat-extract bouillon, saccharose-peptone fluid, dextrose-asparagin fluid, were added to a solution of gelatin no hardening was obtained; the test in every case melted at the same temperature as the control.

The liquefaction appears to proceed slower than the hardening, as the liquefied gelatin can be removed from above the dark medium by carefully washing it with water and the unhardened gelatin by slowly raising the temperature and removing the medium as it liquefies. The portions at the bottom of the tube as well as the top parts of the slope as a rule remain in those cases in which the liquefaction has made a slight headway. It is only when the medium is very dark that the hardening effect can be clearly demonstrated. When the tube is placed in boiling water, the upper portion of the gelatin slope in some cases separates from the glass and appears to be of a horny nature, while the medium at the lower part of the slope seems to be somewhat flabby.

The effect was obtained readily when the microbe had been recently isolated, but after the lapse of nearly a year the bacterium had so far increased the relative production of the liquefying enzyme that one had to adopt means for circumventing the liquefaction. These consisted in growing the bacterium upon glucose-gelatin in the air for a day or two until the darkening had made a slight headway and then putting the culture tube into a Buchner tube. In about three weeks the medium had become uniformly dark and almost black-brown. In some cases the medium at the lower part of the stroke, although apparently soft, had not liquefied, and in placing the culture tube in water at 40° about 0·5 c.c. of the soft medium liquefied; upon this being

removed, the remainder remained firm when the temperature was raised to 100°. In other cases the medium remained firm, dark brown, and did not liquefy.

Bacillus indurans, n.sp.

Shape, etc.—The cells appear as rods with rounded ends. They vary in size; when taken from saccharose-potato-agar and stained, the smallest measure $0{\cdot}5 : 1{\cdot}3\ \mu$, the largest $0{\cdot}6 : 2{\cdot}2\ \mu$. When taken from bouillon, they vary from $0{\cdot}4 : 1\ \mu$ to $0{\cdot}8 : 2\ \mu$. Many of the cells are vacuolated. The bacteria are actively motile, the flagella being numerous and peritrichous. They are negative to the Gram stain. No spores were observed.

Glucose-gelatin plate.—The colonies are flat, glistening and translucent white with a lacerate edge. Microscopically they are translucent and yellowish with central granules and club-shaped structures scattered chiefly in the middle portions. The margin is very finely granular. At a later date, when the colony has sunk into the gelatin, a crumpling, especially at the margins, is observed.

Nutrient agar plate.—The colonies are raised, glistening, translucent white and rounded. When magnified the colonies appear translucent and yellowish with granules scattered around the centre.

Glucose-gelatin stroke.—The growth is narrow, white and flat, with lateral amœboid offshoots. The medium may be very slowly liquefied in the vicinity of the lower end of the stroke. A reddish-mahogany colour appears in the upper layers and slowly diffuses downwards, while the colour deepens. The white culture takes up the colour and becomes a reddish-buff. The medium is rendered insoluble.

Glucose-gelatin stab.—The stab is filiform, the nail-head small, white and flat. There is no apparent liquefaction, and the dark colour slowly diffuses downwards.

Nutrient gelatin stab.—A white flat surface-growth is imbedded in the softened medium. The stab appears as a spiral thread within a tube of soft gelatin.

Nutrient agar stroke.—A smooth, flat, translucent white growth with a smooth edge. A slightly brownish tinge appears in the upper portion of the slope.

Saccharose-potato-agar.—A white, moist, slimy growth.

Potato.—A yellowish, raised, dry-glistening, restricted growth. The medium becomes purplish then brownish.

Bouillon.—The medium becomes turbid and there is formed a faint surface-ring and a coherent sediment. Indol is produced and nitrates are reduced to nitrites.

Milk.—The medium is unaltered.